further understanding of the rotary catalytic mechanism of *pmf*-driven rotation of F_oF_1.

1247-Pos Board B17
Strongly Hydrogen Bonded Water Networks Detected in the Archaerhodopsin-3 Proton Pump
John I. Ogren[1], Erica C. Saint-Clair[1], Sergey Mamaev[1], Joel M. Kralj[2], Adam E. Cohen[2], Kenneth J. Rothschild[1].
[1]Boston Universiy, Boston, MA, USA, [2]Harvard University, Boston, MA, USA.
We examined structural changes of the protein, retinal chromophore and internal water molecules during the photocycle of Archaerhodopsin-3 (AR3), a light-driven proton pump found in Halobrubrum sodemense. This protein, which belongs to the archael rhodopsin family, has a ~75% sequence homology with the more extensively studied bacteriorhodopsin (BR) proton pump from Halobacterium salinarum. Recent interest has focused on AR3 because of its ability to serve both as a high-performance genetically targetable optical silencer of neuronal activity and as a membrane voltage sensor. Low-temperature and rapid-scan time-resolved FTIR-difference spectroscopy revealed that the conformational changes during formation of the K, M and N photocycle intermediates are similar, although not identical, to BR. Positive/negative bands in the region above 3600 cm-1 which have previously been assigned to structural changes of weakly hydrogen bonded internal water molecules were substantially different between AR3 and BR. This included the absence of positive bands recently associated with a chain of proton transporting water molecules in the cytoplasmic channel and a weakly hydrogen bonded water (W401) which is part of a hydrogen-bonded pentagonal cluster located near the retinal Schiff base. However, many of the broad IR continuum changes below 3000 cm-1 assigned to networks of water molecules involved in proton transport through cytoplasmic and extracellular portions of the protein were very similar in AR3 and BR.

1248-Pos Board B18
Investigation of Dopamine Receptor Structure and Function by Structure Prediction and Unnatural Amino Acid Mutagenesis
Ethan B. Van Arnam, Kristina N. McCleary, Fan Liu, Ravinder Abrol, Henry A. Lester, William A. Goddard, III, Dennis A. Dougherty.
California Institute of Technology, Pasadena, CA, USA.
D2 and D4 dopamine receptors were investigated by structure prediction and unnatural amino acid mutagenesis. Active and inactive structures of the D2 dopamine receptor were predicted and the dopamine binding site was modeled. Aromatic residues at and near the binding site of D2 and D4 receptors were interrogated by unnatural amino acid mutagenesis, revealing a network of aromatic-aromatic interactions. Conserved proline residues within five of the D2 receptor's transmembrane helices were also interrogated by unnatural amino acid mutagenesis, revealing a key role for lack of a backbone hydrogen bond donor and importance of a substituent on the backbone N at the Pro5.50 site.

1249-Pos Board B19
Effects of Point Mutations on the Activity of AcrB
Paolo Ruggerone[1], Attilio V. Vargiu[2], Francesca Collu[3].
[1]Universita' di Cagliari, Monserrato (CA), Italy, [2]CNR-IOM SLACS c/o Department of Physics, University of Cagliari, Monserrato (CA), Italy, [3]Department of Chemistry & Biochemistry, University of Bern, Bern, Switzerland.
The tripartite efflux pump AcrAB-TolC is responsible for the intrinsic and acquired multidrug resistance in Escherichia coli. Its active part, the homotrimeric transporter AcrB, is in charge of the selective binding of substrates and energy transduction.
The presence of several phenilalanine residues in the only binding pocket identified by X-ray has inspired an experimental work where the effects of single point Phe->Ala mutations on the MIC and on the efflux of several antibiotics have been determined [1,2]. Concerning the MICs, interestingly, the mutation F610A has been shown to significantly reduce the minimum inhibitory concentration of doxorubicin and many other substrates, although F610 does not appear to interact strongly with them or to be involved in the squeezing mechanism of the binding pocket suggested as preliminary step of the extrusion process. In this work, we assess the impact of the experimental mutations on the functionality of AcrB by means of computational techniques, using doxorubicin and minocycline as substrates. We found that for F610A the compounds slide deeply inside the binding pocket after mutation, increasing the strength of the interaction. During subsequent conformational alterations of the transporter, the substrates were either not extruded from the binding site or displaced along a direction other than the one associated with extrusion. The other mutations are not able to modify the binding affinity of the substrates, which are kept in positions similar to the ones assumed in the wild type protein. Our study indicates how subtle interactions determine the functionality of multidrug transporters, since decreased transport might not be simplistically correlated to decreased substrate binding affinity.
[1] Bohnert, J. A., et al. J. Bacteriol. 2008, 190, 8225-9.
[2] Bohnert, J. A.; Schuster, S.; Szymaniak-Vits, M.; Kern, W. V. PlosOne 2011, 6, e21196.

1250-Pos Board B20
Site Directed Crosslinking Studies of the FepA Transport Mechanism
Kyle J. Moore, Salete M.C. Newton, Phillip E. Klebba.
University of Oklahoma, Norman, OK, USA.
FepA is a TonB dependent transporter in the outer membrane of Gram-negative bacteria that recognizes and internalizes the siderophore ferric enterobactin (FeEnt). FepA is a member of the porin superfamily on the basis of its 22 stranded transmembrane β-barrel. Its 150-residue N-terminal domain resides within and occludes the C-terminal β-barrel domain, suggesting that the former must undergo structural reorganization to allow the transport of FeEnt. Two models potentially explain the process: either the N-domain remains in the β-barrel and rearranges to form a transient pore, or it extrudes from the β-barrel, folded or unfolded. The extent of conformational change in the N-terminus may affect the overall energetic cost of FeEnt transport. To distinguish between these models, we genetically engineered Cys pairs at various sites in FepA and studied their crosslinking both before and during FeEnt uptake. The experiments attempted to either crosslink the N-terminal domain to the β-barrel, or crosslink local folds of the N-terminal domain to itself. Mutant strains of FepA containing the Cys pairs G27C/R126C, A33C/E120C, or L125C/V141C, located on different β-strands in the N-terminus, all required a reductant to allow FeEnt uptake in siderophore nutrition tests, suggesting that oxidation of the Cys pairs to disulfides prevented FeEnt transport. The N-domain/C-domain Cys pairs A138C/427C, A138C/A445C, M77C/T457C, or M77C/E511C readily crosslinked when treated with Cu^{2+} in the presence or absence of FeEnt, implying considerable conformational flexibility of the N-domain *in vivo*. Together these data suggest that the N-domain of FepA has mobility within the β-barrel, and unfolds during transport of FeEnt.

1251-Pos Board B21
Different Purification Approaches for the Integral Membrane Protein EmrE Leads to Biochemical and Biophysical Differences in the Protein
Raymond H. Chew, Raymond J. Turner.
University of Calgary, Calgary, AB, Canada.
EmrE is an integral membrane protein in *Escherichia coli* belonging to a class of multidrug resistance transporters: the Small Multidrug Resistance transporter family (SMR). EmrE effluxes quaternary cationic compounds (QCC) using a proton motive force. Many groups studying this protein utilize a -C-terminal *myc*-eptitope- 6 histidine tag engineered into EmrE for the purpose of protein purification in a detergent-containing aqueous environment. An alternative method for EmrE purification involves using a solvent mixture of chloroform:methanol:water to extract the protein from bacterial membranes. The EmrE isolated using the latter method does not have any tag. As there is different data appearing from different labs, the goal of this research is to compare the protein purified in with the different approaches analyzed under identical conditions. Untagged and tagged EmrE was analyzed and compared by fluorescence spectroscopy. Fluorescence emission spectra of untagged EmrE revealed a lower combined fluorescence intensity of the tyrosine and tryptophan fluorescence compared to tagged EmrE. A red edge excitation shift (REES) effect was also observed for tagged EmrE indicating that there is a population of fluorophores that have a slower rate of solvent relaxation, unlike the fluorophores in untagged EmrE. Functional analysis of the EmrE by QCC ligand-binding (Ethidium, methyl viologen, cetylpyridinium) was investigated using fluorescence quenching binding curves. Untagged EmrE and tagged EmrE binding curves with ethidium were similar, whereas binding of the methyl viologen and cetylpyridinium were different between untagged and tagged EmrE. These results show that the two purification approaches lead to differences in the structural and function states of this integral membrane protein.

1252-Pos Board B22
Expression, Purification and Characterization of Bacterial and Human Translocator Protein 18 kDa (TSPO)
Fei Li, Carrie Hiser, Shelagh Ferguson-Miller.
Michigan State University, East Lansing, MI, USA.
The Translocator Protein 18 kDa (TSPO) has been identified as a key player in cholesterol and porphyrin transport, apoptotic signaling, cancer